

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Wolkers, et al.  
Serial No.: Not Assigned  
Filed: April 5, 2001  
For: THERAPEUTIC  
PLATELETS AND  
METHODS  
Group Art Unit: 1633  
Examiner: Shin-Lin Chen  
Attorney Docket: 6829-60267

**CERTIFICATE OF  
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April 5, 2001

  
Diana R. Castillo

**PRELIMINARY AMENDMENT**

**BOX PATENT APPLICATION**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please enter the following Preliminary Amendment for the subject patent application:

**IN THIS SPECIFICATION**

Please enter the following sentence at page 1, line 8: --- This is a continuation patent application of copending patent application Serial No. 09/501,773, filed February 10, 2000.---

**IN THE CLAIMS**

Please amend Claims 1-3, 6-8, 12, 13, 15, 16, 18, 25, 26, and 32 as follows:

1. (Amended) A dehydrated composition comprising:

freeze-dried platelets selected from a mammalian species and being effectively loaded internally with at least about 10 mM trehalose therein to preserve biological properties during freeze-drying and rehydration, wherein the freeze-dried platelets are rehydratable so as to have a platelet response for the selected mammalian species to at least one agonist.

2. (Amended) The dehydrated composition as in claim 1 wherein the

amount of trehalose loaded inside the freeze-dried platelets is from about 10 mM to about 50 mM.

3. (Amended) The dehydrated composition as in claim 1 wherein the

platelet response to at least one agonist is a response to thrombin in a physiological concentration.

6. (Amended) The dehydrated composition as in claim 1 wherein the

effective loading with trehalose therein of the composition provides substantial stability at ambient temperatures without use of a fixative when protected from moisture until rehydration.

7. (Amended) The dehydrated composition as in claim 1 wherein the effective loading includes incubating platelets at a temperature from about 30° C to less than about 40°C so as to uptake external trehalose via fluid phase endocytosis.

8. (Amended) The dehydrated composition as in claim 1 wherein the mammalian species is human.

12. (Amended) A therapeutic composition useful in transfusion therapy, as a hemostasis aid or for drug delivery, comprising:

platelets having a homogeneously distributed concentration of a therapeutic agent therein, the platelets determinable to have a platelet response to thrombin.

13. (Amended) The therapeutic composition as in claim 12 wherein the determinable response to thrombin is clot formation within about three minutes at 37°C.

15. (Amended) A hemostasis aid, comprising:

freeze-dried human platelets, the platelets being effectively loaded with from about 10 mM to about 50 mM trehalose to preserve biological properties during freeze-drying and rehydration, wherein the platelets are rehydratable so as to have a platelet response to thrombin at physiological concentrations; and,

a biocompatible matrix on which the platelets are carried.

16. (Amended) The hemostasis aid as in claim 15 wherein the platelets are coated on or impregnated in the matrix and are protected from moisture until rehydration.

17. The hemostasis aid as in claim 15 wherein the matrix is a woven or non-woven bandage, wound dressing, or suture.

18. (Amended) A process of preparing a dehydrated composition comprising:

providing platelets selected from a mammalian species, the platelets being effectively loaded with an oligosaccharide therein to preserve biological properties, wherein the loading includes incubating the platelets at a temperature from about 30°C to less than about 40°C with an oligosaccharide solution, the solution having up to about 50 mM oligosaccharide therein, the incubating sufficient to load oligosaccharide inside the platelets in an amount from about 10 mM to about 50 mM;

cooling the loaded platelets to below their freezing point; and,

lyophilizing the cooled platelets.

25. (Amended) The process as in claim 18 wherein the lyophilizing is conducted so as to remove about 95 weight percent of water.

26. (Amended) A process of using a dehydrated composition in wound or burn treatment, comprising:

providing freeze-dried platelets selected from a mammalian species for which treatment is intended, the platelets being effectively loaded with at least about 10 mM of trehalose therein to preserve biological properties; and,

applying the freeze-dried platelets to a wound or burn of the selected mammalian species.

32. (Amended) The process as in claim 29 wherein the prehydration is sufficient to bring the water content of the freeze-dried platelets up to about 50 weight percent.

PLEASE ADD THE FOLLOWING CLAIMS:

33. A dehydrated composition, comprising:

human freeze-dried platelets, the freeze-dried platelets with biological properties preserved during freeze-drying and rehydration by having a composition therein consisting essentially of at least about 10 mM of an oligosaccharide, wherein the freeze-dried platelets are rehydratable so as to have a platelet response to thrombin in a physiological concentration.

34. The composition as in claim 33 wherein the oligosaccharide is in an amount of at least about 10 mM and includes trehalose.

35. The composition as in claim 33 wherein the amount of oligosaccharide loaded inside the freeze-dried platelets is from about 10 mM to about 50 mM.

36. The composition as in claim 33 wherein the oligosaccharide includes trehalose.

37. A process for preparing a dehydrated composition comprising:  
loading internally platelets with from about 10 mM to about 50 mM oligosaccharide to produce internally loaded platelets; cooling the internally loaded platelets to below their freezing point; and lyophilizing the cooled internally loaded platelets.

38. The process of Claim 37 additionally comprising drying the internally loaded platelets prior to said cooling.

39. The process of Claim 38 wherein said drying comprises suspending the internally loaded platelets in a drying solution containing a water replacing molecule.

40. The process of Claim 39 wherein said drying solution comprises up to about 100 mM of an oligosaccharide.

41. The process of Claim 37 wherein said cooling comprises cooling the internally loaded platelets to a temperature below about -32°C.

42. The process of Claim 37 wherein said loading comprises incubating platelets at a temperature greater than about 25°C.

43. The process of Claim 37 wherein said loading is without a fixative.

44. A dehydrated composition produced in accordance with the process of Claim 37.

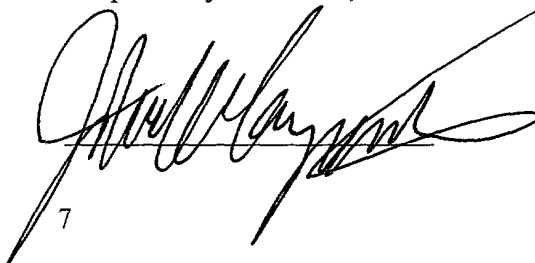
45. A dehydrated composition comprising freeze-dried platelets selected from a mammalian species and internally comprising at least about 10 mM of an oligosaccharide.

Remarks

The foregoing amendment to the claims, along with the additional claims which have been added, patentably define various embodiments of the present invention.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version With Marking to Show Changes Made.**"

Respectfully submitted,



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**Version With Marking to Show Changes Made**

**In the Claims**

Claims 1-3, 6-8, 12, 13, 15, 16, 18, 25, 26, and 32 have been amended as follows:

1. (Amended) A dehydrated composition, ~~useful for mammalian therapy,~~ comprising:

~~substantially shelf-stable~~ freeze-dried ~~platelets~~ selected from ~~the a~~ mammalian species ~~for which therapy is intended, the freeze-dried platelets and~~ being effectively loaded internally with at least about 10 mM trehalose therein to preserve biological properties during freeze-drying and rehydration, wherein the freeze-dried platelets are rehydratable so as to have a ~~normal~~ platelet response for the selected mammalian species to at least one agonist.

2. (Amended) The dehydrated composition as in claim 1 wherein the amount of trehalose loaded inside the freeze-dried ~~blood~~ platelets is from about 10 mM to about 50 mM.

3. (Amended) The dehydrated composition as in claim 1 wherein the ~~normal~~ platelet response to at least one agonist is a response to thrombin in a physiological concentration.

6. (Amended) The dehydrated composition as in claim 1 wherein the effective loading with trehalose therein of the composition is substantially provides substantial shelf stability stable at ambient temperatures without use of a fixative when protected from moisture until rehydration.

7. (Amended) The dehydrated composition as in claim 1 wherein the effective loading includes incubating platelets at a temperature from ~~greater than~~ about 25° 30° C to less than about 40°C so as to uptake external trehalose via fluid phase endocytosis.

8. (Amended) The dehydrated composition as in claim 1 wherein the mammalian species is platelets ~~are human platelets.~~

12. (Amended) A therapeutic composition useful in transfusion therapy, as a hemostasis aid or for drug delivery, comprising:

platelets having a homogeneously distributed concentration of a therapeutic agent therein, the platelets determinable to have a ~~normal~~ platelet response to thrombin.

13. (Amended) The therapeutic composition as in claim 12 wherein the determinable ~~normal~~ response to thrombin is clot formation within about three minutes at 37°C.

15. (Amended) A hemostasis aid, comprising:

~~substantially shelf-stable freeze-dried~~ human platelets ~~selected from the mammalian species for which therapy is intended~~, the platelets being effectively loaded with from about 10 mM to about 50 mM trehalose to preserve biological properties during freeze-drying and rehydration, wherein the platelets are rehydratable so as to have a normal platelet response to ~~at least one agonist~~ thrombin at physiological concentrations; and,

a biocompatible matrix on which the platelets are carried.

16. (Amended) The hemostasis aid as in claim 15 wherein the platelets are coated on or impregnated in the matrix and are protected from moisture until rehydration.

18. (Amended) A process of preparing a dehydrated composition, ~~useful for therapy to a mammal~~, comprising:

providing platelets selected from ~~the~~ a mammalian species ~~for which therapy is intended~~, the platelets being effectively loaded with an oligosaccharide therein to preserve biological properties, wherein the loading includes incubating the platelets at a temperature from ~~greater than~~ about 25 30°C to less than about 40°C with an oligosaccharide solution, the solution having up to about 50 mM oligosaccharide therein, the incubating sufficient to load oligosaccharide inside the platelets in an amount from about 10 mM to about 50 mM;

cooling the loaded platelets to below their freezing point; and,

lyophilizing the cooled platelets.

25. (Amended) The process as in claim 18 wherein the lyophilizing is conducted at a temperature below about  $-32^{\circ}\text{C}$  and removes so as to remove about 95 weight percent of water.

26. (Amended) A ~~therapeutic~~ process of using a dehydrated composition in wound or burn treatment, comprising:

providing freeze-dried platelets selected from a mammalian species for which ~~therapy~~ treatment is intended, the platelets being effectively loaded with at least about 10 mM of trehalose therein to preserve biological properties; and,

applying the freeze-dried platelets to a wound or burn of the selected mammalian species.

32. (Amended) The process as in claim 29 wherein the prehydration is sufficient to bring the water content of the freeze-dried platelets up to ~~between about 35 weight percent to about 50 weight percent~~.

The following claims have been added:

33. A dehydrated composition, comprising:

human freeze-dried platelets, the freeze-dried platelets with biological properties preserved during freeze-drying and rehydration by having a composition therein consisting essentially of at least about 10 mM of an oligosaccharide, wherein the

freeze-dried platelets are rehydratable so as to have a platelet response to thrombin in a physiological concentration.

34. The composition as in claim 33 wherein the oligosaccharide is in an amount of at least about 10 mM and includes trehalose.

35. The composition as in claim 33 wherein the amount of oligosaccharide loaded inside the freeze-dried platelets is from about 10 mM to about 50 mM.

36. The composition as in claim 33 wherein the oligosaccharide includes trehalose.

37. A process for preparing a dehydrated composition comprising:  
loading internally platelets with from about 10 mM to about 50 mM oligosaccharide to produce internally loaded platelets; cooling the internally loaded platelets to below their freezing point; and lyophilizing the cooled internally loaded platelets.

38. The process of Claim 37 additionally comprising drying the internally loaded platelets prior to said cooling.

39. The process of Claim 38 wherein said drying comprises suspending the internally loaded platelets in a drying solution containing a water replacing molecule.

40. The process of Claim 39 wherein said drying solution comprises up to about 100 mM of an oligosaccharide.

41. The process of Claim 37 wherein said cooling comprises cooling the internally loaded platelets to a temperature below about -32°C.

42. The process of Claim 37 wherein said loading comprises incubating platelets at a temperature greater than about 25°C.

43. The process of Claim 37 wherein said loading is without a fixative.

44. A dehydrated composition produced in accordance with the process of Claim 37.

45. A dehydrated composition comprising freeze-dried platelets selected from a mammalian species and internally comprising at least about 10 mM of an oligosaccharide.